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Reversal of Impaired Renal Function in Rats with Streptozotocin-induced Diabetes by Transplantation of Isolated Pancreatic Islets: Failure in Preventing The Progress of Glomerulosclerosis

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Abstract

In an attempt to evaluate the reversibility of the diabetic nephropathy, the glomerular filtration rate (GFR) and the renal blood flow (RBF) were measured in rats with streptozotocin (STZ)-induced diabetes and in rats with corrected carbohydrate metabolism by isolated pancreatic islets isografting two months (group A) and four months (group B) after STZ administration. Decreases in both GFR and RBF were observed as early as two months after the onset of severe glucose intolerance. ($P<0.01$) While GFR measured by the inulin clearance method remained at rather moderately impaired levels, RBF measured by the microsphere method showed a progressive decrease ($P<0.01$) during the time of observation for eitht months. Those rats that received isolated pancreatic islet transplantation showed significant improvements in both GFR ($P<0.01$ in both groups A and B) and RBF ($P<0.05$ in group A and $P<0.01$ in group B). Histological studies, however, revealed that, though slowed, the sclerotic change in the glomeruli was progressive in spite of the correctd carbohydrate metabolism. These results with limited reversibility of the kidney changes initiated by the diabetic state emphasize the importance of the optimum regulation of blood glucose levels starting at early stages of diabetes.

Introduction

Diabetes, one of the leading causes of death, has resulted in the definitely lower survivorship of the diabetic population[1]. Recently reported prospective studies have proved a strong correlation between the development of the diabetic complications and the duration of clinically recognized diabetes[9,21], and it has been announced by the American Diabetes Association that these diabetic complications can be prevented by precise regulations of the carbohydrate metabolism with a suggestion of the necessity of developing better means to achieve control than those at present

Key words: Islet transplantation, Diabetic nephropathy, Glomerulosclerosis, Tubular vacuolization and reversibility.
索引語：膵島移植，糖尿病性腎病変，糸球体硬化，尿細管空泡変性，可逆性.
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available[5]. In recent years, techniques of transplantation of isolated pancreatic islets have been developed[2] and proved to have a potential capability to normalize the impaired carbohydrate metabolism in experimental animals[2,11]. It has also been reported that diabetic changes in the kidney were successfully reversed by strict regulations or normalizations of the blood glucose levels either by pancreatic transplantation[13,14,26,28] or by careful administrations of insulin[17,18,20]. The present study was designed to evaluate the reversibility of the diabetic changes in the kidney in terms of glomerular permeability changes, changes in renal hemodynamics and their correlations to histological observations under an "ideal normalization" of severely impaired carbohydrate metabolism in rats with STZ-induced diabetes by a currently available methology of isolated pancreatic islet transplantation.

Materials and methods

Animals and induction of diabetes : Highly inbred Wistar rats, sex-week-old male, supplied by Kyoto University Animal Facility, were fed on regular rat chow and water ad libitum. After an assessment of the health and growth condition for three weeks, STZ in a dose of 70 mg/kg body weight was administered intravenously[8]. The animals were defined as diabetic only when ; 1) Fasting blood glucose levels were higher than 300 mg/dl, 2) Urinary outputs exceeded 100 ml/day with (3+) glucose excretion by Tes Tape® and 3) Decrease in body weight continued for an observation period of three weeks. The following animals composed the present study : group A ; 10 rats that received pancreatic islet transplantation two months after the onset of the glucose intolerance (TP A), and group B ; 10 rats kept in the diabetic state for four months before the correction of the impaired carbohydrate metabolism by islet isografting (TP B). For each of the two groups, non-islet-transplanted diabetic (10 for group A and 9 for group B) and non-STZ-treated healthy (10 for groups A and B respectively) littermates served as controls.

Pancreatic islet preparation : Islets for each transplantation were obtained from eight rats weighing 200 to 250 g, by the collagenase digestion method follwed by the Ficoll-dencity-gradient separation[23]. For all procedures of the islet preparation, 0.5% (w/v) bovine serum albumin, 10U/ml Trasylol® and 100 U/ml penicillin kalium G were added to Hanks' balanced solution (HBS). Acinar-tissue-disrupted and sharp-scissors-minced pancreatic tissues were digested by collagenase (Sigma type IV, 100 mg for pancreatic tissues from 8 rats) at 37°C for approximately 25 minutes. The pancreatic digests were washed with HBS and then mixed with 27%(w/v) Ficoll-Hanks' solution. The tissue-Ficoll mixture was placed in the bottom of 50 ml centrifuge tubes, and 23%, 20% and 10% Ficoll-Hanks' solutions were layered. After a centrifugation at 800 rpm, 1,500 rpm and 3,000 rpm for 5 minutes respectively, the islets were recovered from the interface between 23% and 20% Ficoll layers. Contaminating large ductal debris were removed by sieving the islet suspension through a 300 micrometer stainless steel mesh screen, and small acinar fragments and excess-digested islets were removed by repeating settling and suspending in warm HBS. More than 1,000 intact islets were constantly obtained from 8 rats, and this amount of islets suspended in 1 ml HBS was injected into the portal vein without causing a trouble of portal hypertension (Fig. 1).

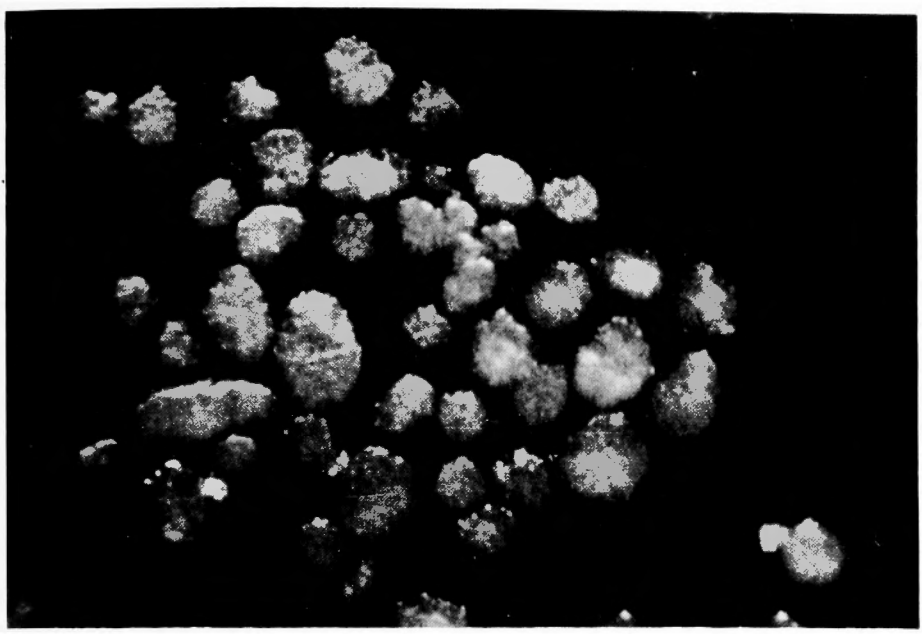

Fig. 1. Isolated and purified pancreatic islets. More than 1,000 islets from eight young doners were injected into the portal vein without causing portal hypertension. (Original magnification: ×40)

Intravenous glucose tolerance tests (IV-GTT): IV-GTTs were performed two weeks prior to the clearance and microsphere studies. The animals were fasted overnight, and under a very light anesthesia with ether, oneshot glucose load (500 mg/kg by 50% glucose solution)[6] was given through a jugular vein and blood samples were obtained from the another. Samples were placed in icecold immediately. Blood glucose was measured by the oxidase method using an auto-analyser and serum immunoreactive insulin (IRI) was determined by the two antibody technique with appropriate human standards.

Clearane and microsphere studies : Clearance studies were performed in a mannner described elsewhere[10], two months (group A) and four months (group B) after the islet injection into the protal vein. Briefly, under light anesthesia with ether, a polyethylene catheter (PE 50) was fixed in a jugular vein for infusion purpose and another catheter (PE 20) was placed in the right femoral artery to obtain blood samples. The bladder was cannulated through a suprapubic cystostomy with a silastic tubing. The urethra was ligated at the vesical neck. Animals were immobilized in plastic rat holders, and clearance studies were conducted in conscious animals at room temperature. Following the priming dose of 5 mg/kg body weight, a sustaining infusion of inulin (10 mg/ml) was given at a rate of 0.0457 ml/min. After an equilibration period of 60 minutes, three control clearance periods were obtained. Clearance of inulin was calculated from the standard formula : Clearance = (urinary concentration / plasma concentration) × urine flow rate. Inulin was measured by the anthrone method[7]. At the completion of the clearance study, another PE 20 catheter was placed in the left ventricle through the left carotid artery and the animals were rested for another 60 minutes before the microsphere technique was applied. 15 micron carbon particles

labelled with Sr-85 (3M BRAND TRACER MICROSPHERE) were suspended in dextran containing saline (2×10^6/ml) and 0.1ml of the solution was flushed into the left ventricle using a 1ml tuberculin syringe[4]. The sample blood was collected from a femoral artery for one minute. Animals were killed by an intracardiac injection of 1 ml KCl solution (1.0 M), and kidneys were removed, weighed and separated into the outer cortex, the inner cortex and the medullar area with sharp scissors. The samples were weighed and their radioactivities were counted using a crystal scintillation counter.

Statistical analysis : Results were presented as mean ± standard deviation (SD). The statisical significance of difference was assessed by Student's t test and P values less than 0.05 were considered significant.

Histological studies : Tissues taken at the animal death were formalin-fixed, paraffin-embedded and stained with hematoxylin and eosin.

Results

General observations : Fig. 2 shows body weight changes of rats in goup B. The STZ injection caused rapid reduction of body weights, which did not return to the pretreatment levels spontaneously. Following the intraportal injection of more than 1;000 isolated pancreatic islets, urinary glucose excretion was reduced, eventually becoming trace to negative in 24 to 36 hours

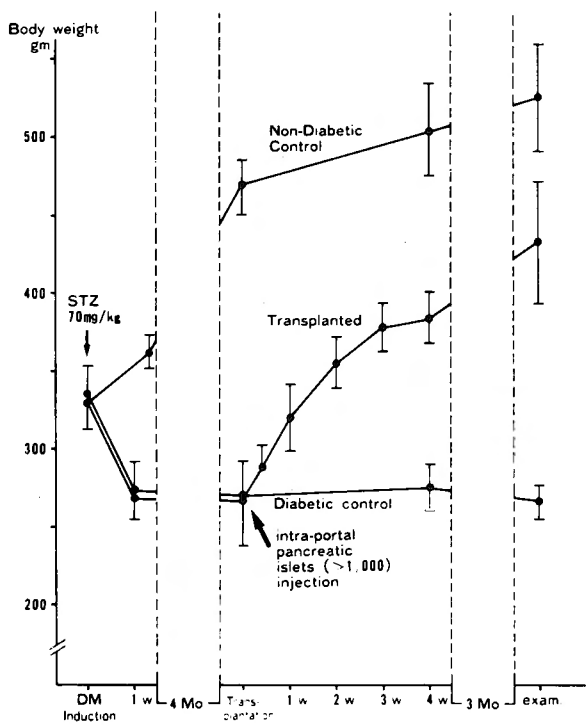

Fig. 2. Body weight changes of rats in group B. Note the marked wight loss caused by STZ administration, and rapid catch-up gains after receiving pancreatic islet isografts.

when tested with Tes-Tape. Urine volumes were reduced from more than 100 ml/day to less than 30 ml/day within a week. Rapid catch-up weight gains which often reached up to 50 g during the first week of the posttransplantation period were observed, but the body weights did not reach to those of non-diabetic controls. Diabetic cataracta, which developed during the third month of diabetes (11 of 15 candidates for the islet transplantation in group B), disappered in 2 to 3 months after the restoration of normal carbohydrate metabolism. The IV-GTT showed normalized glucose disappearance curves, and marked hyperinsulinemias in both group A and group B rats (Fig. 3).

Clearance studies : GFRs were decresed as early as two months after the onset of diabetes. The decrease in GFR, however, was not progressive and GFRs remained at rather moderately impaired levels (Fig. 4). By the islet transplantation, GFRs were significantly improved in all rats in group A and group B. ($P<0.01$) The P value between the GFRs of the age matched, non-diabetic rats and those that received islet isografts was less than 0.01 in group B, while P value was greater than 0.01 in group A, suggesting that the reversibility of GFR might have been affected by the prolonged duration of the carbohydrate intolerance in group B rats.

Renal blood flow studies : RBFs were also decreased two months after the onset of diabetes, and the decrease in RBF was progressive (Fig. 5). By islet transplantation, RBFs were signifi

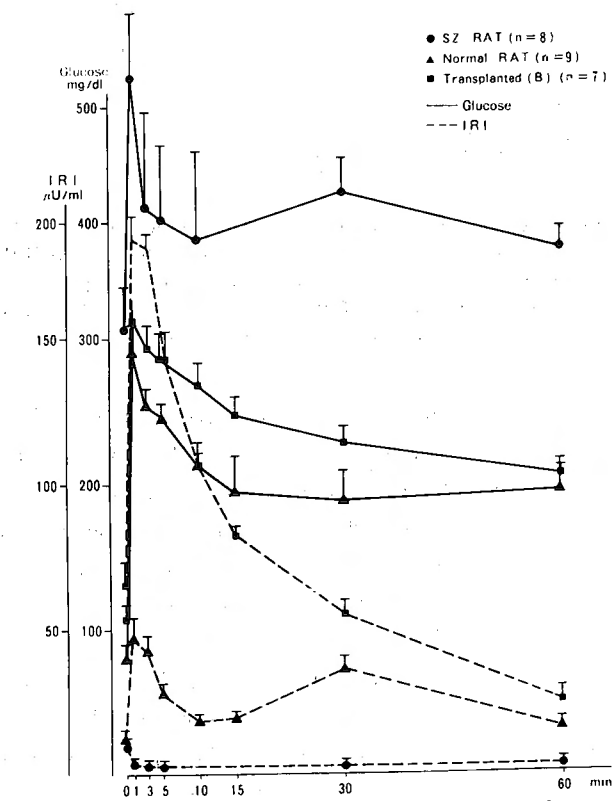

Fig. 3. Intravenous glucose tolerance tests showed normalized glucose disappearance curves accompanied by marked hyperinsulinemias in rats that received islet injection into the potal vein.

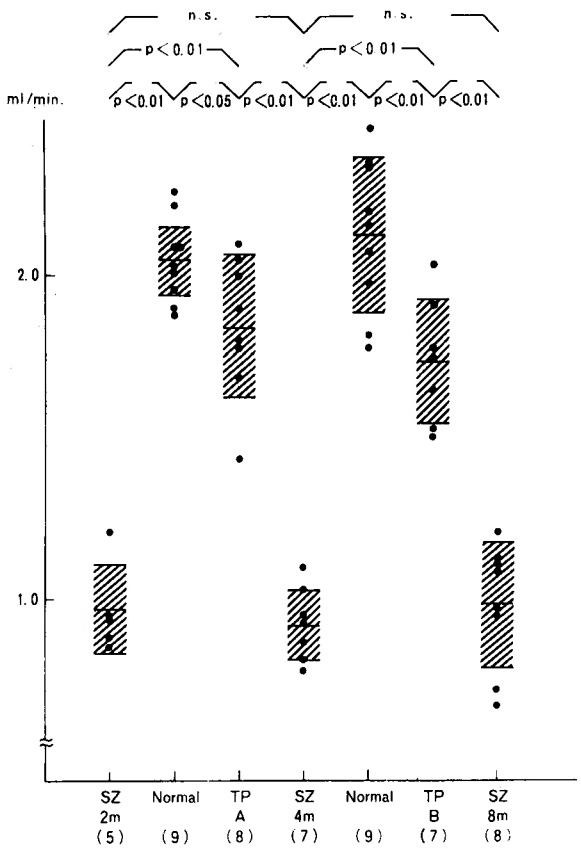

Fig. 4. The inulin clearance study showed significant reduction of GFR in diabetic rats both in group A and B. Note that those decrease in GFR were not progressive with the prolongation of the diabetic state. Improvements in GFR were achieved by restorations of the carbohydrate metabolism, though GFRs never reached to those in non-diabetic control rats.

cantly improved and no statistical difference was found between the RBFs of transplanted rats and those of age-matched healthy controls in group A. Although RBFs in diabetes-corrected rats in group B were smaller than those in age-matched, non-diabetic controls ($P<0.01$), the P value became greater than 0.05 when blood flows per gram of the outer cortex were culcurated (Fig. 6). This was mainly through the fact that in rats that received islet isografts after certain periods of the impaired carbohydrate metabolism (group B), the kidneys were severely contracted and had only small amounts of renal cortex.

Histological findings: A most prominent finding in the kidney of rats with STZ-induced diabetes was the vacuolization of the proximal tubules (Fig. 8, 10 and 12). The lining cells were occupied by clear cytoplasma losing their nuclei. One of the typical glomerular changes was the difuse sclerosis, which was observed as early as two months after the onset of diabetes (Fig. 7) and was more prominent in group B diabetic rats (Fig. 10). Another consistent finding in group B diabetic rats was the presence of glycogen-like material in the distal tubules. In proportion to

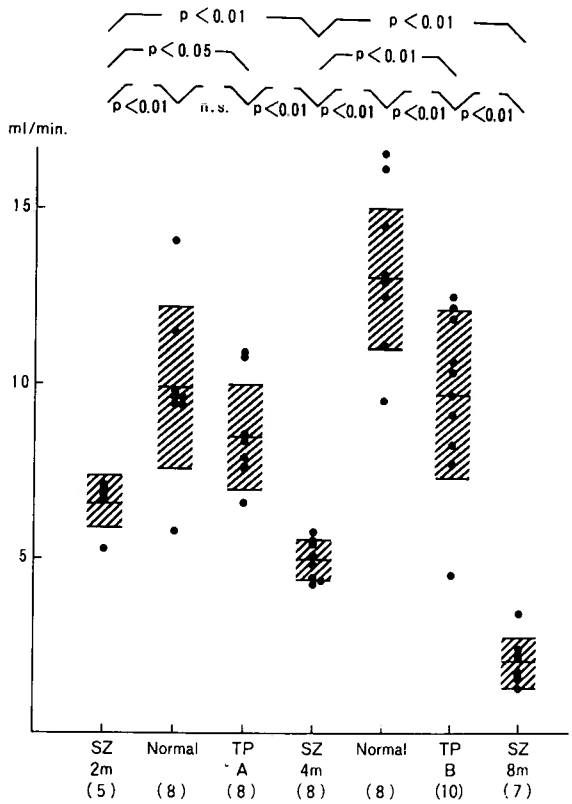

Fig. 5. Microsphere measurements showed progressive decrease in the blood flow to the kidney with the prolongation of the diabetic state. Islet transplantation successfully reversed the impaired renal blood flow.

the severity of glomerular sclerosis, the space between the capillaries and the Bowman's capsule was diminished (Fig. 7 and 10). In rats that received islet transplantation, the vacuolization of the proximal tubules had disappeared, or, if any, they were only regional with a lesser severity (Fig. 9, 11 and 12). In spite of the restoration of the normal glucose metabolism, however, the sclerotic change in the glomeruli was progressive when compared to the findings in pretransplantation controls, though it was apparently less severe than the findings in the age-matched, diabetic controls (Fig. 7 and 9, 8 and 11). Figure 13 shows microspheres found in severely sclerotic glomeruli in a rat diabetic for eight months.

Discussion

The introduction of insulin therapy and the development of medical care have brought the remarkably improved life expectancy of diabetics[1]. As a result, however, the diabetic complications[22] has become the serious burden on those patients, causing the profound, confusing and long-standing controversy regarding the responsible ; independently inherited genetically linked tissue disorder or hyperglycemia per se. It was not until 1970s that hyperglycemia per se was

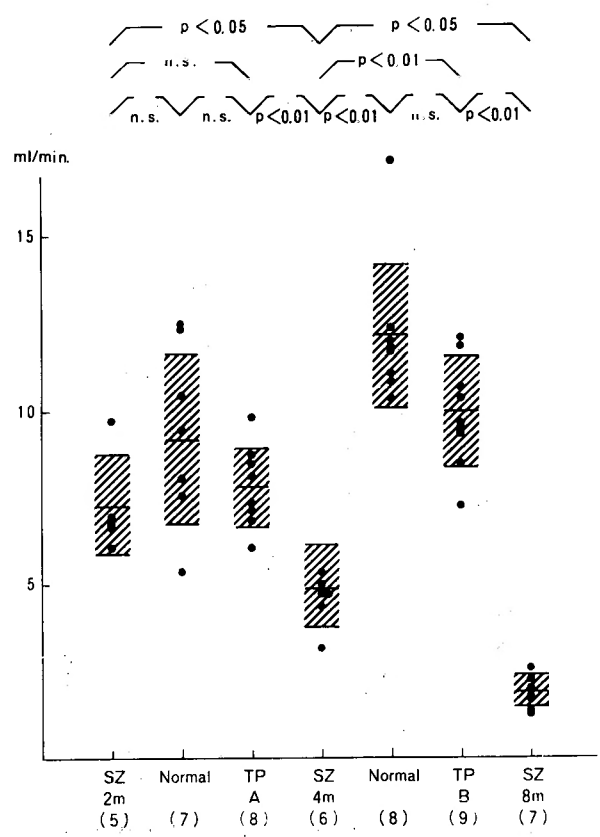

Fig. 6. The blood flow calculated per gram of the outer cortex showed no statistical significance of difference between in rats that received islet transplantation and in non-diabetic controls.

widely accepted to be the responsible to the diabetic complications. In 1972, BALLINGER et al[2]. reported a success in grafting isolated pancreatic islets and showed a disappearance of diabetic cataracta in the rat. In 1974, LEE et al.[12] found that diabetic glomerular changes did develop in normal kidneys transplanted in diabetic rats and that diabetic glomerular changes were reversed upon transplantation of kidneys from diabetic rats into normal recipients. In 1974, MAUER et al.[13] reported that successful pancreatic islet transplantation in rats resulted in regression or arrest of the diabetic glomerular lesions. In 1975, JOB et al.[9], and in 1978, PIRART[21] reported prospective studies in human diabetics revealing close relations between the blood glucose levels and the development of diabetic complications. MAUER et al.[14] extended their work to the reversibility of diabetic changes in the kidney using a technique of pancreatic islet transplantation in rats. WEIL et al.[28] studied renal changes in rats using pancreatic organ transplantation technique. More recently, WEBER et al.[26] reported effects of islet transplantation on renal function analyzing the urinary components.

In the present study using rats and a technique of transplantation of isolated pancreatic islets, inulin clearance studies were combined with microsphere measurements of the renal blood flow,

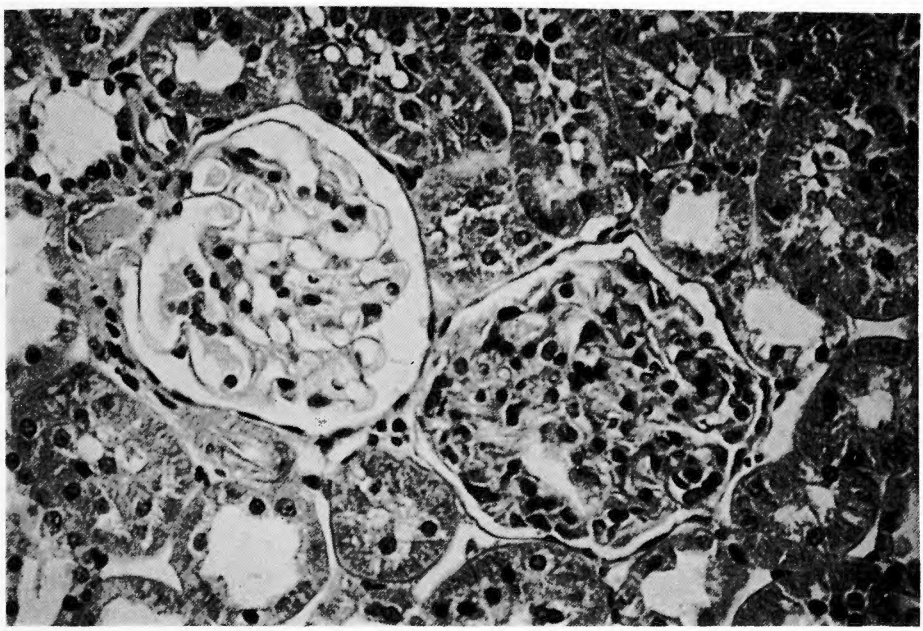

Fig. 7. Glomeruli from a rat with diabetes for two months, showing co-existence of normal or hyperfunctioning glomeruli and sclerotic glomeruli. Hematoxylin-eosin stain, ×100.

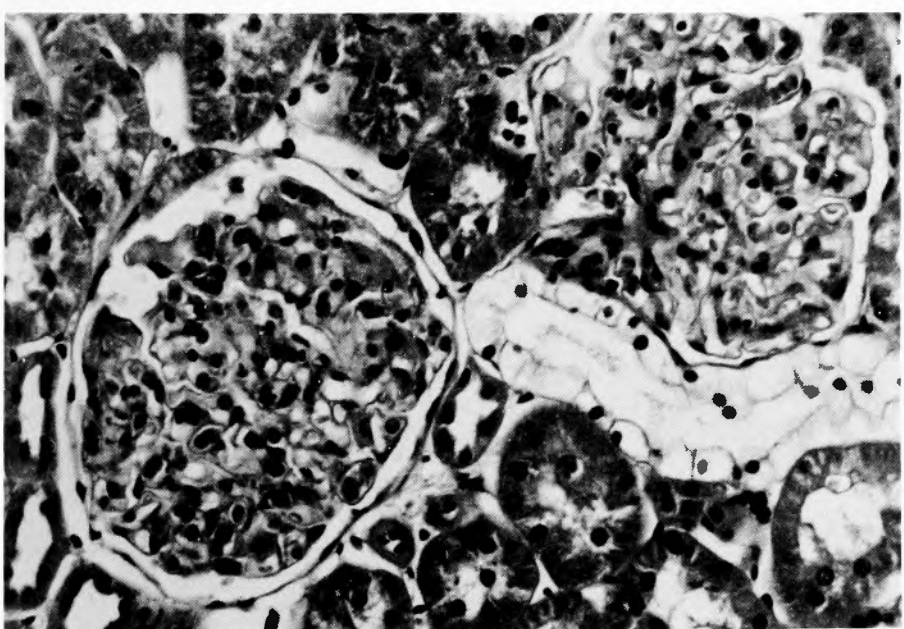

Fig. 8. Sclerosis in the glomerulus and vacuolizations of the proximal tubules in a rat with diabetes for four months. Hematoxylin-eosin stain, ×100.

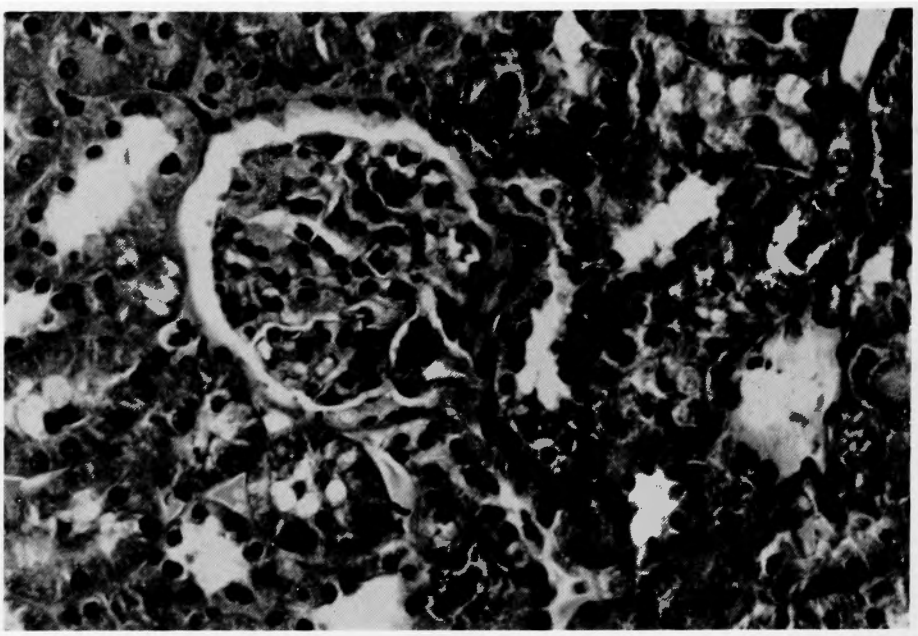

Fig. 9. In rats that received pancreatic islet transplantation after two months of diabetes, the glomerular functions were preserved but the sclerotic changes were more severe when compared with the findings in the pretransplantation control rats (Fig. 7). Hematoxylin-eosin stain, ×100.

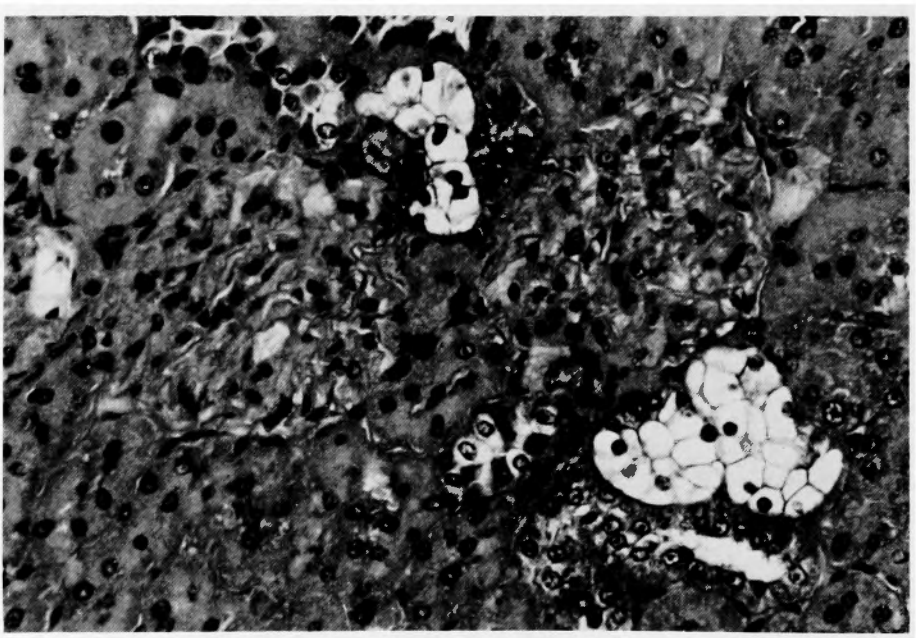

Fig. 10. Severe glomerulocslerosis and tubular vacuolizations in a rat diabetic for eight months. Hematoxylin-eosin stain, ×100.

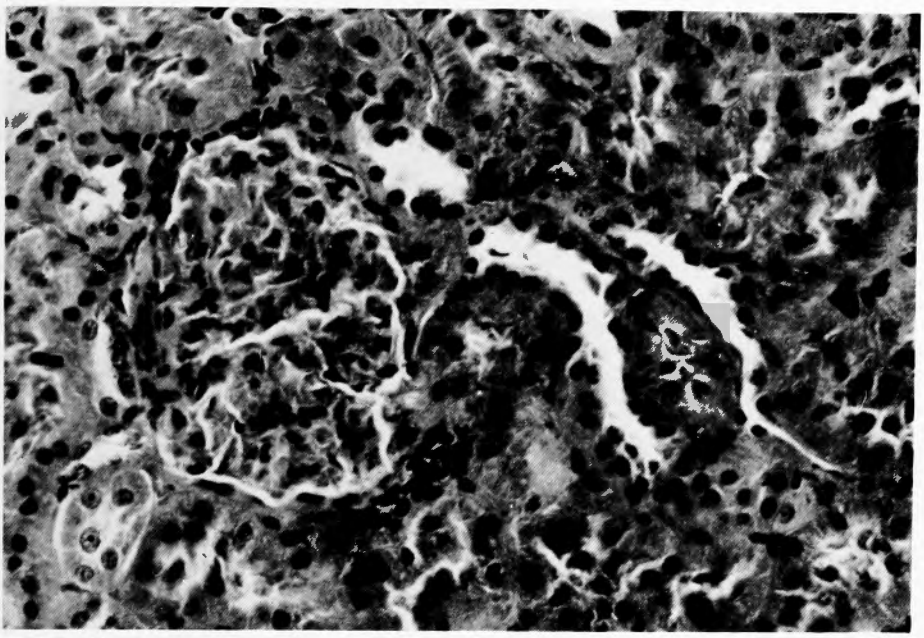

Fig. 11. In rats that received islet transplantation after four months of diabetes, the tubular vacuolization had disappeared. Note that the sclerotic changes were more severe when compared with the findings in pretransplantation control rats (Fig. 8). Hematoxylin-eosin stain, ×100.

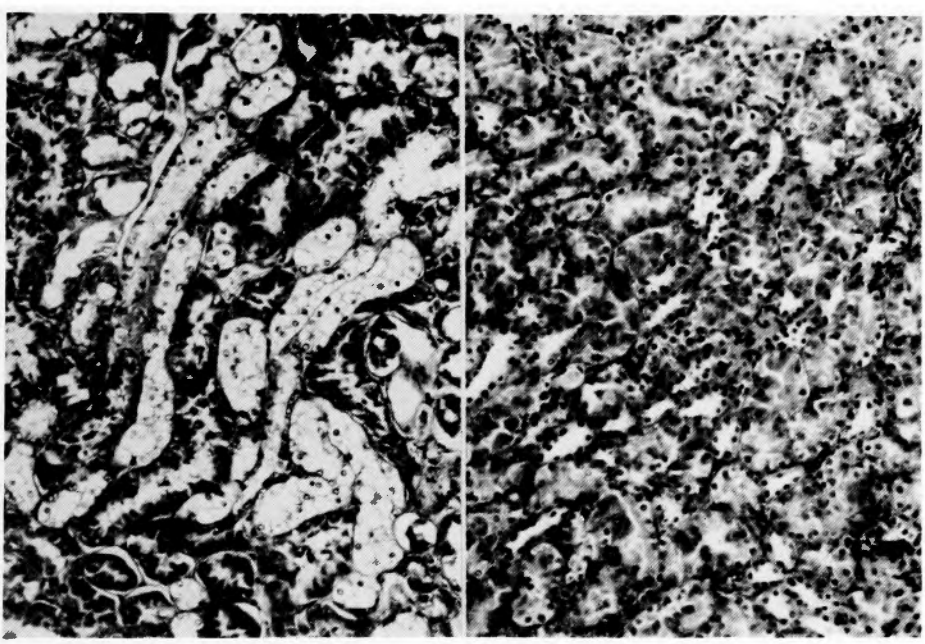

Fig. 12. Tubular vacuolizations found at fourth month of diabetes (left) were successfully reversed by pancreatic islet transplantation (right). Hematoxylin-eosin stain, ×50.

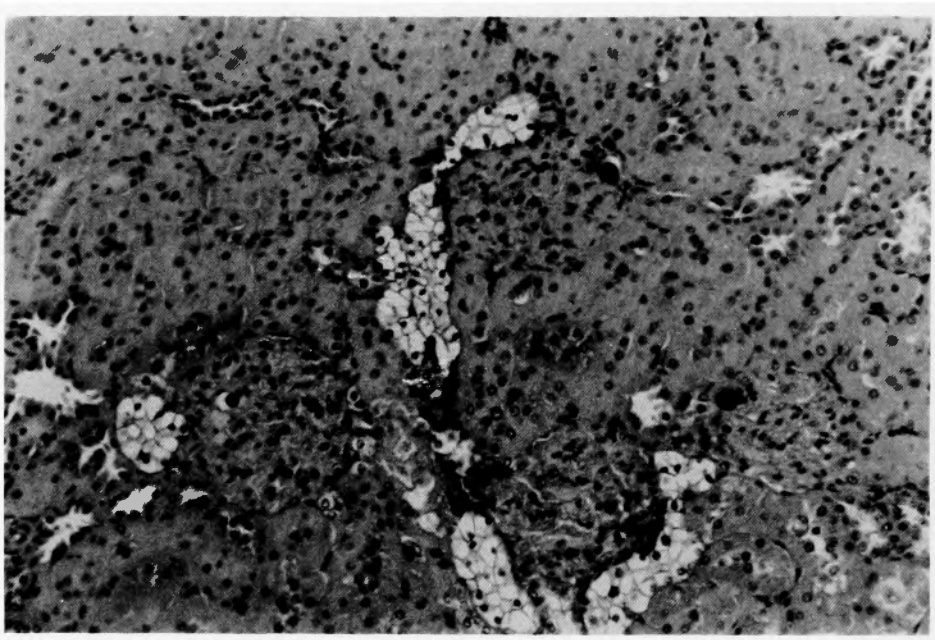

Fig. 13. Microsheres found in severely sclerotic glomeruli in a rat with diabetes for eight months, suggesting a possibile erroneous evaluation of the renal function by microsphere measurement of the blood flow.
Hematoxylin-eosin stain, ×50.

to evaluate the factors determinating the renal changes in diabetes, namely permeability changes in the glomeruli and changes in the renal hemodynamics, and their correlations to histological findings at correction of the carbohydrate metabolism.

As shown in Fig. 2, the catch-up gain of the body weights after the islet transplantation was remarkable. Shortness of the interval between the the islet transplantation and the liberation from urinary glucose excretion as well as the disappearance of the diabetic cataracta proves the completeness of the reversal of the impaired carbohydrate metabolism. Our results were confirmative with the following findings reported by others : 1) Intraportal injection of isolated pancreatic islets can completely ameliorate the STZ-induced diabetes[11], 2) Islet injections into the portal vein were resulted in marked hyperinsulinemias with normalized glucose disappearance curves at IV-GTT[25], 3) Kidney changes in STZ-induced diabetic rats including the diffuse glomerulosclerosis, the tubular vacuolization and the deposit of glycogen-like materials in the distal tubules were progressive[27], and 4) Restoration of normal carbohydrate metabolism by pancreatic islet transplantation did successfully reverse the diabetic kidney changes in terms of reversal of the tubular vacuolization[13].

In our results, there was a clear-cut discrepancy between the behaviors of GFR and RBF. Under the diabetic state, RBF was progressively decreased, while GFR remained at moderately impaired levels. This is probably through progressive increases in single nephron GFRs or the development of "leaky glomeruli"[20], since the kidneys showed progressive contractions decreasing the number of functioning glomeruli. The decrease in RBF seemed to be rather

proportional to the histological findings of the progressive glomerulosclerosis. By the islet transplantation, both RBF and GFR were improved significantly. However, these functional parameters never reached to the levels of non-diabetic controls in group B rats, reflecting the macroscopic observation of the contracted kidney. The result of microsphere measurements caliculated per gram of the outer cortex in rats that received islet isografts ($P>0.05$ to non-diabetics in group B) was somewhat misleading, since microspheres were found even in those gomeruli that were apparently not functioning due to sclerosis (Fig. 13).

In contrast to the report by MAUER et al.[13,14] that the progressive mesangial thickening was reversed after successful pancreatic islet transplantation, our results with severe disorder of carbohydrate metabolism for certain durations suggest that, though retarded, the sclerotic change of the glomeruli per se was not reversed by the restoration of normal carbohydrate metabolism. These different results in the reversibility may mainly be through the difference of the severity of diabetes employed in the experiments. Our severely diabetic model resulted in an early development of glomerulosclerosis (Fig. 2), and a limited reversibility. These findings of limited reversibility were also suggested by others[3,18,24], indicating that the once-established sclerotic changes would not be reversed even by an "ideal normalization" of the diabetic state by pancreatic islet transplantation. Moreover, our results that the sclerotic change in the glomeruli in rats with normalized carbohydrate metabolism after certain periods of diabetes was in more severe conditions when compared to the findings in the pretransplantation control rats would suggest that once the sclerosis were initiated by diabetes, this sclerotic change could be accerelated by the sclerotic change itself through factors including the renal hypertension as was suggested by MAUER et al. in rats with Goldblatt hypertension[15] and in those with unilateral nephrectomy[16]. Mogensen also experienced that satisfatory control of blood pressure postponed the state of uremia in diabetic patients[19]. Thus, our results with limited reversibility and failure in preventing the progress of the sclerotic change in the glomeruli emphasize the impotatnce of the optimum regulation of the blood glucose levels starting at early stages of diabetes.

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糖尿病患者の平均余命は非糖尿病者のそれに比して絶対的に短く，その原因として様々な合併症が挙げられる．その一つである糖尿病性腎病変について，その可逆性を，ストレプトゾトシン (STZ) 糖尿病ラットにおいて，糸球体濾過率 (GFR) 及び腎血流量 (RBF) の変化を指標として光顕所見と比較しつつ，現在考えられる最も完全なる糖尿病コントロールの手段としての膵ランゲルハンス氏島（膵島）移植を行い検討した．

同一週令の体重約 250 g のウイスター・ラットに STZ (70 mg/kg, iv) を投与し，糖尿病ラットを作製した．糖尿病誘発後，2ケ月目（A群）および4ケ月目（B群）に，同系のラット（体重 200-250 g）よりコラーゲナーゼ消化法により得た分離膵島 1,000 個以上を門脈内に注入し，糖尿病状態を解消した後，更らに各々2ケ月および4ケ月を経過させ，それぞれの時点における腎機能を，イヌリン・クリアランス法による GFR 及びマイクロ・スフェアーを用いての RBF により評価し，光顕所見と比較検討した．

STZ 投与により，空腹時血糖は 300 mg/dl 以上，尿量は 100 ml/日以上となり，個体の体重増加は停止した．膵島移植により個体は直ちに体重増加を示し，50 g/週に達することもあった．尿糖は24-36時間以内に消失し，尿量も1週間以内に 20-30 ml/日に減じた．糖負荷試験では正常の血糖曲線とともに高インスリン血症を示した．

糖尿病誘発後，GFR, RBF ともに有意に減じたが，GFR の減少が非進行性であったのに対して，RBF は進行性に減少した．膵島移植により，GFR, RBF ともに有意に改善したが，A群の RBF を除いては，いずれも正常コントロール群の値に復するには至らなかった．

組織学的には，進行する糸球体の性硬化，近位尿細管の空泡変性，及び遠位尿細管への糖原様物質の蓄積が見られた．膵島移植により，尿細管の空泡変性，糖原様物質の蓄積については改善乃至消失が見られるが，糸球体の硬化はその進行は遅延させられたものの，硬化所見そのものは進行した．このことは，起因された糸球体の硬化は糖尿病状態から開放されても進行するものであることを示唆し，糖尿病初期における血糖管理の重要性を示す．また，血流測定に用いたマイクロ・スフェアーが硬化した糸球体にも多く見られたことは，マイクロ・スフェアー法による血流測定は必らずしもその病態を反映したものではないことを示唆する．